Supporting Information for:

Methanospirillum respiratory mRNA biomarkers correlate with hydrogenotrophic methanogenesis rate during growth in competition with organochlorine-respiring *Dehalococcoides* in a mixed culture.

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Supplemental Materials and Methods

Analysis of Organic Acids

Experimental samples were filter-sterilized via 0.2 μm PTFE coated syringe filter and stored at -20^{o}C in auto-sample vials until IC analysis. Each sample was run via isocratic 5-mM sodium hydroxide gradient through an AS-1100 column (Dionex) with a total run time of 30 min (5 min ramp to 80 mM sodium hydroxide at the end of each run). For each experimental set, organic acid standards were run for butyrate, formate, propionate and acetate ranging from 1 μM to 10mM in filtered basal salts medium (BSM, (*1*)). The detection limit for most organic acids was 10 μM, with the exception of formate which had a 1 μM detection limit.

Hydrogen addition experiments

Hydrogen additions were performed in two different modes: batch addition to headspace or via continuous flux through diffusion tubing. Hydrogen levels were kept above one hundred times the reported K_s (0.5 μM) for methanogenesis in this culture (*2*), and were maintained with bulk hydrogen additions to the headspace. Alternately, a slow rate of hydrogen addition was generated in serum vials through the diffusion of hydrogen across low-density polyethylene (LDPE) 3/8-in. OD × 1/4-in. ID × 0.062-in. wall tubing (Freelin-Wade 1J-074). Construction and

use of hydrogen diffusion tubes was performed as described previously for oxygen permeability experiments (*3*), substituting hydrogen for oxygen. In brief, tubing was cut to equivalent lengths of approximately 6.5 cm and sealed with barbed-end PVC plugs, maintaining a 5 cm internal length (volume 1.6 mL). This internal volume was filled with either 66 μmoles of H_2 or N_2 (as a control). Abiotic control samples were used to calculate rates of hydrogen diffusion in basal salts media (BSM) for each hydrogen addition experiment.

Methyl fluoride inhibition experiments

To a subset of cultures continuously fed butyrate, methyl fluoride (MF) was added as a selective inhibitor of acetoclastic methanogenesis (*4*). MF at a partial pressure of 1 kPa has previously been shown to selectively inhibit acetoclastic methanogenesis without affecting syntrophic interactions in an anaerobic mixed culture including acetogenic, sulfate-reducing and fermentative bacteria (*5*). MF (Sigma) was measured in cultures via GC-FID (using standard chloroethene run conditions described) and maintained at a minimum partial pressure of 1 kPa, but below 5 kPa in microcosm headspace. This is due to the observation that partial pressures greater than 5 kPa may inhibit hydrogenotrophic methanogenesis (*5*).

Methanospirillum Primer Design

Degenerate primers for methanogen hydrogenases were used to obtain *M. hungatei* -specific sequences from the Donna II mixed community via clone libraries as described previously (*6*). Hydrogenase subgroups targeted were the energy-conserving hydrogenase (EchA), the methyl-viologen reducing hydrogenase subunit D (MvrD) and the nickel-iron hydrogenase large subunit (F_{420}-reducing, FrcA). Cloned sequences generated in this analysis matched the *M. hungatei* JF-1 genome with 90- 99 percent nucleotide identity, with the exception of Ech which only produced *D. mccartyi* str. 195 sequences. All sequences were later

confirmed in the Donna II metagenome (IMG-M/ER) and were utilized to design quantitative PCR primers for Donna II *M. hungatei* biomarker targets (Table S2) using PrimerQuest available through IDT (www.idtdna.com). Primers were also tested with JF-1 pure culture DNA extracts and cloned amplicons (data not shown). Metagenomic sequencing of this community suggests high homology and synteny between the Donna II *M. hungatei* population and *M. hungatei* JF-1, a strain consisting of four ribosomal gene copies (JGI-MER website).

Microarray Design and Processing

The microarray designed for this experiment was an Agilent Technologies© two-color, 15k, 60 mer, 8 plex array. The specific designs of the probes utilized a modified method provided by the eArray© software suite (*7*). The probe set includes all *Dehalococcoides mccartyi* (formerly *ethenogenes*) str. 195 predicted open reading frames, non-protein encoding RNA transcripts (rRNAs, tRNAs), community member 16S rDNA, *M. hungatei* hydrogenase sequences, and a luciferase control. The designed probes were searched using the Basic Local Alignment Search Tool (BLAST) (*8*) against both the National Center for Biotechnology Information (NCBI) nucleotide collection and the assembled mixed community metagenome (IMG). The microarray platform is uploaded and freely available at the NCBI Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/).

50 mL of liquid culture samples were centrifuged at 14190 *g*. The centrifuged sample was split into 8 individual RNA extractions with each sample following the RNeasy Mini Kit (Qiagen) extraction previously outlined. The 8 distinct RNA extractions were recombined on the spin filter before the first RW1 buffer wash. The Superscript I DNAse RNA cleanup, amino-allyl cDNA formation, cDNA cleanup, and cDNA labeling with Cy3 or Cy5 followed the method outlined (*7*). The quality and quantity of the RNA was determined using the RNA 6000 Nano

assay on an Agilent 2100 bioanalyzer (Agilent Technologies). The quantity of resulting cDNA was determined by using the Quant-IT OliGreen ssDNA Assay Kit (Invitrogen). A common control RNA pool sampled from the main Donna II reactor after 3 days of starvation was labeled with Cy3, which served as the reference dye.

For each experiment, Cy5 labeled cDNA from the mixed community mRNA pool was hybridized against an aliquot of common control of Cy3 labeled cDNA from 3-day starved culture. The hybridization, washing, and scanning of the microarray samples was performed by the Cornell University Microarray Core Facility (http://cores.lifesciences.cornell.edu/brcinfo/) and followed the methods outlined by the manufacturer (*7*). The general procedure mixed 25 µl (~400 ng) of the labeled cDNA sample with 25 µl 2x Gene Expression (GEx) Hybridization Buffer HI-RPM (8), hybridized the sample to the microarray slide at 65° C for 17 hours, washed with GEx Wash Buffer 1 and 2 (*7*) at room and elevated (37° C) temperatures, and scanned with an Agilent Technologies Scanner G2505C with a 5 µm resolution.

Microarray image analysis was conducted using Agilent Feature Extraction 10.5 Image Analysis Software. The Feature Extraction Software was also utilized to perform a within-array modified LOESS normalization between the Cy5 and Cy3 signals, to calculate a log ratio between the Cy5 and Cy3 channels, and to calculate a modified Student t-test p-value between the Cy5 and Cy3 signal distributions (*7*). The more detailed treatment the Agilent Feature Extraction employed can be found in the user manual (*10*). Replicate spots for the same probe (ranging from 6-20 spots/probe) were geometrically averaged. The raw and normalized data is freely available at the NCBI GEO database.

Supplemental Tables

Table S1. Experimental parameters for continuous feed and batch fed Donna II sub-cultures used to study protein mRNA biomarkers. Replicate reactors listed for each experiment including information on feeding regime, respiration rates and hydraulic residence time.

Experiment Title (Continu-ous Feed)	Replicate Name	Chloroethene electron Acceptor (EA)	EA feeding rate (µeeq/L-hr)	Electron Donor (ED)	ED:EA (H2 equiva-lents)	Length of Experiment (days)	Dehalo-respiration rate (µeeq/L-hr)	Methano-genesis rate (µeeq/L-hr)	Average Hydrogen Conc. per reactor (µM nominal)	Average Aqueous Hydrogen Conc. (µM)	Hydraulic Residence time (days)
Decay	Time Zero 3	-	-	-	-	0	-	-			-
	DecayA1	-	-	-	-	7	-	1.2	0.60	0.019	-
	DecayB1	-	-	-	-	7	-	1.5	0.70	0.022	-
	DecayB2	-	-	-	-	3	-	2.4	0.99	0.031	-
Butyrate	B1	-	-	Butyrate	-	1	-	281	0.33	0.10	14
	B2	-	-	Butyrate	-	1	-	277	0.38	0.12	14
Butyrate MF	BMF1	-	-	Butyrate	-	1	-	74	0.44	0.14	14
	BMF2	-	-	Butyrate	-	1	-	54	0.32	0.10	14
	Control1	-	-	-	-	1	-	-	0.02	0.01	-
	Control2	-	-	-	-	1	-	-	0.03	0.01	-
Hydrogen	HH1	-	-	H_2	-	1	-	173	51	16	16.7
	HH2	-	-	H_2	-	1	-	170	53	16	16.7
	HH3	-	-	H_2	-	1	-	157	51	16	16.7
	H2A1	-	-	H_2	-	1.5	-	0.5	1.4	0.05	12
	H2A2	-	-	H_2	-	1.5	-	2.5	3.3	0.10	12
PCE Hydrogen	H2PB1	PCE	10	H_2	0.5	1.5	9.7	1.1	0.52	0.016	12
	H2PB2	PCE	8.8	H_2	0.5	1.5	7.3	1.4	0.93	0.029	12
PCE Butyrate High	HiP1	PCE	259	Butyrate	3	1	140	124	2.0	0.062	1.25
	HiP2	PCE	231	Butyrate	3.4	1	133	127	2.0	0.062	1.25
	HiP3	PCE	280	Butyrate	2.8	1	167	172	2.3	0.072	1.25
PCE Butyrate High Low	High PSS (HHL3)	PCE	183	Butyrate	4.3	7	137.8	390	5.2	0.16	10
	HLL1	PCE	4.9	Butyrate	1.4	7	4.9	94	1.2	0.036	40

	Low PSS (HLL2)	PCE	4.7	Butyrate	1.4	7	4.8	92	1.9	0.062	40
	HLL3	PCE	5.9	Butyrate	1.1	7	5.9	119	1.9	0.061	40
PCE Half Butyrate	PHB1	PCE	104	Butyrate	0.29	7	85	387	0.52	0.016	11
	PHB2	PCE	45	Butyrate	0.66	7	39	466	0.93	0.029	11
	PHB3	PCE	106	Butyrate	0.28	7	97	389	0.37	0.011	11
PCE Lactate	PLL1	PCE	48	Lactate	0.81	7	45	516	0.98	0.031	10
	PLL2	PCE	39	Lactate	0.51	7	37	358	1.3	0.042	10
PCE No Donor	PnfyN1	PCE	3	-	-	7	1.3	0.6	1.0	0.033	40
	PnfyN2	PCE	3	-	-	7	1.3	0.7	0.99	0.031	40
	PnfyF1	PCE	4.5	fermented yeast extract	-	7	4.8	38.7	0.85	0.027	40
	PnfyF2	PCE	5.4	fermented yeast extract	-	7	6.5	51.8	1.4	0.043	40
	PnfyY1	PCE	4.9	yeast extract	-	7	4.5	42.4	1.1	0.035	40
	PnfyY2	PCE	4.7	yeast extract	-	7	4.3	39.5	1.1	0.033	40
PCE Butyrate	P3A1	PCE	25	Butyrate	1.5	4	25	469	3.0	0.091	10
	P3A2	PCE	22.6	Butyrate	1.7	4	23	394	2.8	0.085	10
	P3B1	PCE	4.3	Butyrate	1.9	4	4.5	331	4.1	0.13	10
	P3B2	PCE	4.8	Butyrate	1.7	4	4.9	357	5.4	0.17	10
	P3C1	PCE	0.9	Butyrate	2.3	4	1	329	4.3	0.13	10
	P3C2	PCE	0.9	Butyrate	2.2	4	0.9	317	2.6	0.079	10
TCE Butyrate	T3A1	TCE	51	Butyrate	3.2	4	34	473	1.5	0.047	10
	T3A2	TCE	35	Butyrate	2.7	2	23	368	1.0	0.032	10
	T3B1	TCE	10	Butyrate	3.5	4	6.9	169	0.69	0.022	10
	T3B2	TCE	11	Butyrate	3.3	4	7.3	205	1.1	0.035	10
	T3C1	TCE	2.2	Butyrate	3.9	4	1.5	73	0.98	0.031	10
	T3C2	TCE	2.1	Butyrate	4.1	4	1.4	92	0.81	0.026	10
DCE Butyrate	D3A1	DCE	30	Butyrate	2.2	1	30	185	6.6	0.21	10
	D3A2	DCE	32	Butyrate	2.6	4	32	101	1.9	0.058	10
	D3B1	DCE	8.9	Butyrate	2	4	8.9	165	1.1	0.035	10
	D3B2	DCE	8.2	Butyrate	2.2	4	8.2	158	6.6	0.21	10

	D3C1	DCE	2.3	Butyrate	1.9	4	2.3	58	4.0	0.13	10
	D3C2	DCE	2.3	Butyrate	1.8	4	2.3	82	1.7	0.055	10

Experiment Title (Batch Feed)	Replicate Name	Electron Acceptor (EA)	Total EA fed (µM)	Electron Donor (ED)	ED:EA (H2 equivalents)	Length of Experiment (day)	Dehalo-respiration products (µeeq/L)	Methane Production (µeeq/L)	Peak Hydrogen Conc. (µM nominal)	Peak Hydrogen Conc. aqueous (µM)	Hydraulic Residence time
Batch	Time Zero 1	PCE	220	Butyrate	2	7	946	9438	0.17	0.053	70
	TS 2	PCE	220	Butyrate	2	7	1126	9215	0.11	0.036	70
	TS 3	PCE	220	Butyrate	2	7	952	5102	0.15	0.047	70
	Time Zero 2	PCE	220	Butyrate	2	7	1320	5359	0.15	0.047	70

Table S2. RNA and DNA biomarker targets. Gene loci based on *Dehalococcoides mccartyi* str. 195 or *Methanospirillum hungatei* str. JF1, along with gene name and annotation based on information from IMG (http://img.jgi.doe.gov.) are listed. Primer sequences used for quantitative PCR reported along with annealing temperature and reference.

Organism	**Gene Locus**	**Gene Name**	**Annotation/ IMG term**	**Primer Sequence**	**Annealing temp for qPCR**	**Reference**
Dehalococcoides mccartyi str.195	DET_DE16S	16S rRNA	16S ribosomal RNA	GGAGCGTGTGGTTTAATTCGATGC (sense) GCCCAAGATATAAAGGCCATGCTG (anti-sense)	60^{O}C	(*11*)
	DET0110	HupL	[Ni/Fe] hydrogenase, group 1, large subunit (EC:1.12.99.6)	TGACGTTATTGCAGTAGCTGAGT (sense) CACACCATAGCTGAGCAGGTT (anti-sense)	55^{O}C	(*11*)
	DET1545	DET 1545	reductive dehalogenase, putative	ATACTTACCGGTCAAGGGCGTTAG (sense) ATGGTCACGATGTTCCTGGGTAAG(anti-sense)	60^{O}C	(*11*)
Methanospirillum hungatei	MHUN_R001 MHUN_R027 MHUN_R068 MHUN_R072	16S rRNA	16S ribosomal RNA	AGTAACACGTGGACAATCTGCCCT (sense) ACTCATCCTGAAGCGACGGATCTT (anti-sense)	60^{O}C	(*6*)
	MHUN2332	FrcA	nickel-dependent hydrogenase, large subunit, Coenzyme F420-reducing hydrogenase, alpha subunit (EC 1.12.98.1)	AGGTCAGCCTTGAAGATGCAGACT (sense) TTCTTGAACTGAACCAGACGGGCA (anti-sense)	60^{O}C	This publication
	MHUN1839 MHUN1842	MvrD	methyl-viologen-reducing hydrogenase, delta subunit, F420-non-reducing hydrogenase, subunit D (EC 1.8.98.1)	TGTTCGTATGCAGGTGCTGACCTT (sense) ACCATCTGCACCCTCAACAAATGC (anti-sense)	60^{O}C	(*6*)

Table S3. Proteins identified in Donna II mixed culture Shotgun proteomics that are assignable to *Methanospirillum hungatei* sequences in either the publically available genomes or available metagenomic sequences. . Each gene locus is relative to the *Methanospirillum hungatei* JF-1 genome (http://img.jgi.doe.gov) with corresponding sequence description, and enzyme commission number. ProtScores are determined by Protein Pilot 2.0^{TM} software and are indicative of sum of contributing high confidence peptides (see methods for further details). G.O. assignments and E.C. numbers generated with the software Blast2GO (*12*). * indicates protein best hit was a homolog in the Donna II metagenome.

ProtScore Unused	ProtScore Total	%Protein Cov(95)	Gene Locus (JF-1)	Sequence Description	a.a.seq. length	Gene Ontology	Enzyme Codes
43.47	43.47	26.3	Mhun_2513	hypothetical protein Mhun_2513	847	C:ribosome; F:structural constituent of ribosome; P:translation	EC:3.6.5.3
23.05	25.06	22.2	Mhun_2148	methyl-coenzyme M methylreductase alpha subunit	567	P:methanogenesis; F:metal ion binding; F:coenzyme-B sulfoethylthiotransferase activity	EC:2.8.4.1
22.25	22.25	58.1	Mhun_2147*	methyl-coenzyme M methylreductase gamma subunit	222	F:structural constituent of ribosome; C:small ribosomal subunit; P:translation; F:coenzyme-B sulfoethylthiotransferase activity; F:rRNA binding; P:methanogenesis	EC:3.6.5.3; EC:2.8.4.1
18.12	18.12	15.4	Mhun_0996	tpr repeat-containing protein	634	F:binding	
16.76	16.83	10.4	Mhun_2023	formate dehydrogenase alpha subunit	685	F:formate dehydrogenase activity; C:formate dehydrogenase complex; P:oxidation reduction; F:electron carrier activity; P:transcription; P:formate metabolic process; F:molybdenum ion binding	EC:1.2.1.2
15.99	15.99	16.2	Mhun_2257*	Coenzyme F420-dependent N(5),N(10)-methenyltetrahydromethanopterin	328	F:coenzyme F420-dependent N5,N10-methenyltetrahydromethanopterin reductase activity; P:oxidation reduction	EC:1.5.99.11

14.98	14.98	28.2	Mhun_2255	methylenetetrahydromethanopterin dehydrogenase	280	C:cytoplasm; F:tetrahydromethanopterin S-methyltransferase activity; C:vesicle membrane; P:oxidation reduction; P:one-carbon metabolic process; F:methylenetetrahydromethanopterin dehydrogenase activity; F:ferredoxin hydrogenase activity; C:integral to membrane; P:methanogenesis; P:sodium ion transport	EC:2.1.1.86; EC:1.5.99.9; EC:1.12.7.2
14.4	15.7	17.7	Mhun_2144*	methyl-coenzyme M methylreductase beta subunit	435	P:cysteine metabolic process; F:pyridoxal phosphate binding; F:cysteine desulfurase activity; F:transaminase activity; F:coenzyme-B sulfoethylthiotransferase activity; P:methanogenesis	EC:2.8.1.7; EC:2.6.1.0; EC:2.8.4.1
10.6	10.6	12.6	Mhun_2332*	coenzyme f420-reducing hydrogenase alpha subunit	358	F:FAD binding; C:membrane; P:oxidation reduction; F:iron-sulfur cluster binding; F:ferredoxin hydrogenase activity; F:nickel ion binding; F:coenzyme F420 hydrogenase activity	EC:1.12.7.2; EC:1.12.98.1
10.58	10.83	10.8	Mhun_0128	chaperone protein	610	P:auxin biosynthetic process; P:protein folding; P:response to stress; P:oxidation reduction; F:ATP binding; F:unfolded protein binding; F:2-alkenal reductase activity	EC:1.3.1.74
10.1	10.1	15.6	Mhun_2175*	tetrahydromethanopterin s-methyltransferase subunit h	340	F:tetrahydromethanopterin S-methyltransferase activity; P:one-carbon metabolic process	EC:2.1.1.86
9.94	16.56	9.2	Mhun_2021	formate dehydrogenase alpha subunit	686	F:formate dehydrogenase activity; C:formate dehydrogenase complex; P:oxidation reduction; F:electron carrier activity; P:transcription; P:formate metabolic process; F:molybdenum ion binding	EC:1.2.1.2

8.64	8.64	10.7	Mhun_1272	carbon monoxide dehydrogenase catalytic subunit	628	P:oxidation reduction; C:cytoplasm; F:carbon-monoxide dehydrogenase (acceptor) activity; P:generation of precursor metabolites and energy; F:4 iron, 4 sulfur cluster binding; F:nickel ion binding	EC:1.2.99.2
6.39	6.39	3.6	Mhun_1838	4fe-4s ferredoxin iron-sulfur binding domain protein	671	F:iron-sulfur cluster binding; F:electron carrier activity; F:CoB--CoM heterodisulfide reductase activity; F:FAD binding; P:methanogenesis; P:tRNA processing	EC:1.2.7.1
6.27	6.7	7.6	Mhun_2549	thermosome	552	P:protein folding; F:unfolded protein binding; F:ATP binding; P:auxin biosynthetic process	
6.18	6.18	10.4	Mhun_0521	abc transporter tungsten-binding protein	307	F:transporter activity; C:integral to membrane; C:membrane; P:molybdate ion transport; F:hydrolase activity; P:transport; F:molybdate transmembrane-transporting ATPase activity; F:molybdate ion transmembrane transporter activity; C:plasma membrane	EC:2.7.4.3
5.88	9.27	8.3	Mhun_2332	coenzyme F420 hydrogenase subunit alpha	469	F:FAD binding; P:oxidation reduction; F:iron-sulfur cluster binding; F:ferredoxin hydrogenase activity; C:plasma membrane; F:nickel ion binding; F:coenzyme F420 hydrogenase activity	EC:1.6.5.3
5.83	5.83	11.1	Mhun_1835	4Fe-4S ferredoxin iron-sulfur binding domain protein	388	F:4 iron, 4 sulfur cluster binding; P:oxidation reduction; F:metal ion binding; F:electron carrier activity; F:formylmethanofuran dehydrogenase activity	EC:1.6.5.3
5.77	5.77	10.8	Mhun_2329	coenzyme F420-reducing hydrogenase subunit beta	288	F:acetate kinase activity; F:ATP binding; C:cytoplasm; P:organic acid metabolic process; P:phosphorylation	

5.16	5.16	8.8	Mhun_1837	heterodisulfide reductase subunit b	296	P:methanogenesis; P:oxidation reduction; F:CoB--CoM heterodisulfide reductase activity; P:cofactor metabolic process	
4.47	4.47	8.3	Mhun_1990	formylmethanofuran dehydrogenase subunit c	266	F:electron carrier activity; F:iron-sulfur cluster binding	EC:4.2.1.33
4.41	4.41	23.8	Mhun_0131	ferritin dps family protein	164	F:ferric iron binding; P:oxidation reduction; F:oxidoreductase activity; P:cellular iron ion homeostasis	EC:6.1.1.20
4.25	4.25	11.4		flagellin	175	F:electron carrier activity; F:iron-sulfur cluster binding	EC:2.7.6.1
4.04	4.04	11.6	Mhun_1554*	beta-lactamase domain protein	216	F:metal ion binding; F:signal transducer activity; P:oxidation reduction; F:hydrolase activity; F:oxidoreductase activity; P:signal transduction; F:electron carrier activity; F:FMN binding	
4	4	8.1	Mhun_2330	coenzyme F420-reducing hydrogenase gamma subunit	262	F:quinone binding; F:electron carrier activity; F:NADH dehydrogenase (ubiquinone) activity; P:transport; F:nickel ion binding; F:4 iron, 4 sulfur cluster binding; F:coenzyme F420 hydrogenase activity; F:FAD binding; P:electron transport chain	
3.96	3.96	8.3	Mhun_0085	aliphatic sulfonate binding protein precursor	350	F:signal transducer activity; P:signal transduction; P:regulation of transcription, DNA-dependent	
3.94	3.95	3.7	Mhun_0148	pas pac sensor protein	299	F:formylmethanofuran dehydrogenase activity; P:oxidation reduction; P:methanogenesis	
3.92	3.92	7.9	Mhun_1988	formylmethanofuran dehydrogenase subunit b	443	C:cytoplasm; P:auxin biosynthetic process; F:peptidase activity; P:protein metabolic process; F:ATPase activity; F:DNA binding; F:protein binding; F:nuclease activity; F:ATP binding; P:nucleotide-excision repair	

3.43	3.48	2.6	Mhun_1813*	formate dehydrogenase alpha subunit	688	C:intracellular; F:formate dehydrogenase activity; F:electron carrier activity; C:formate dehydrogenase complex; F:molybdenum ion binding; P:oxidation reduction; P:formate metabolic process; F:transcription factor activity; P:regulation of transcription, DNA-dependent	EC:2.7.7.4; EC:3.6.5.1; EC:3.6.5.2; EC:3.6.5.3; EC:3.6.5.4
0	3.47	2.6	Mhun_1813	formate dehydrogenase alpha subunit	688	C:intracellular; F:formate dehydrogenase activity; F:electron carrier activity; C:formate dehydrogenase complex; F:molybdenum ion binding; P:oxidation reduction; P:formate metabolic process; F:transcription factor activity; P:regulation of transcription, DNA-dependent	EC:2.4.2.19
0.03	1.73	1.3	Mhun_1833	formate dehydrogenase alpha subunit	687	F:formate dehydrogenase activity; C:formate dehydrogenase complex; P:oxidation reduction; F:electron carrier activity; P:transcription; P:formate metabolic process; F:molybdenum ion binding	
0.03	2.49	1.3	Mhun_3238	formate dehydrogenase alpha subunit	687	F:formate dehydrogenase activity; C:formate dehydrogenase complex; P:oxidation reduction; F:electron carrier activity; P:transcription; P:formate metabolic process; F:molybdenum ion binding	EC:2.7.7.4; EC:3.6.5.1; EC:3.6.5.2; EC:3.6.5.3; EC:3.6.5.4
3.27	3.28	2.1	Mhun_2022	formate dehydrogenase beta subunit	383	F:pseudouridine synthase activity; F:iron-sulfur cluster binding; F:formate dehydrogenase activity; F:electron carrier activity; F:RNA binding; P:oxidation reduction; F:pseudouridylate synthase activity;	

						P:tRNA pseudouridine synthesis	
3.26	3.27	1	Mhun_1406	methyl-accepting chemotaxis sensory transducer	1091	F:translation elongation factor activity; P:two-component signal transduction system (phosphorelay); F:sulfate adenylyltransferase (ATP) activity; P:peptidyl-histidine phosphorylation; P:regulation of transcription, DNA-dependent; P:translational elongation; F:ATP binding; P:signal transduction; F:GTPase activity; F:two-component sensor activity; C:cytoplasm; C:membrane; F:GTP binding	
3.26	3.27	2.4	Mhun_1592	translation elongation factor ef-subunit alpha	425	F:translation elongation factor activity; P:two-component signal transduction system (phosphorelay); F:sulfate adenylyltransferase (ATP) activity; P:peptidyl-histidine phosphorylation; P:regulation of transcription, DNA-dependent; P:translational elongation; F:ATP binding; P:signal transduction; F:GTPase activity; F:two-component sensor activity; C:cytoplasm; C:membrane; F:GTP binding	EC:1.4.1.2
2.77	2.91	4.43	Mhun_2174	tetrahydromethanopterin s-methyltransferase subunit a	248	P:mRNA catabolic process; F:3'-5'-exoribonuclease activity; F:RNA binding; F:polyribonucleotide nucleotidyltransferase activity; C:mitochondrion; P:RNA processing	
2.57	29.47	17.1	Mhun_2263	hypothetical protein Mhun_2263	862	P:oxidation reduction; F:oxidoreductase activity; F:electron carrier activity; F:transition metal ion binding	EC:6.3.4.3
0.02	1.54	4.7	Mhun_1311	rubrerythrin	190	P:oxidation reduction; F:oxidoreductase activity; F:electron	

						carrier activity; F:transition metal ion binding	
2.39	2.39	5.6	Mhun_0613	peptidase m50	377	F:oxidoreductase activity; F:iron-sulfur cluster binding; P:oxidation reduction	
2.36	2.36	2.7	Mhun_2840	surface layer protein	963	C:light-harvesting complex; P:oxidation reduction; F:L-erythro-3,5-diaminohexanoate dehydrogenase activity; P:protein-chromophore linkage; C:chloroplast; F:zinc ion binding	
2.21	2.21	2.1	Mhun_2610	phosphoenolpyruvate synthase	762	F:structural constituent of ribosome; C:small ribosomal subunit; P:translation	
2.18	2.18	4.3	Mhun_0248	periplasmic binding protein	375	F:iron-sulfur cluster binding; F:formate dehydrogenase activity; F:electron carrier activity; P:pseudouridine synthesis; P:oxidation reduction; F:lyase activity	
2.17	2.53	2.2	Mhun_1814	formate dehydrogenase beta subunit	414	F:iron-sulfur cluster binding; F:formate dehydrogenase activity; F:electron carrier activity; P:pseudouridine synthesis; P:oxidation reduction; F:lyase activity	
2.16	2.16	10.4	Mhun_2063	protein	212	C:cytoplasm; P:auxin biosynthetic process; F:P-P-bond-hydrolysis-driven protein transmembrane transporter activity; F:metal ion binding; C:plasma membrane; P:protein import; P:intracellular protein transmembrane transport; F:ATP binding; P:protein targeting; P:protein secretion	EC:3.6.3.6; EC:3.6.3.14; EC:5.99.1.3
2.15	2.15	1.8	Mhun_1989	formylmethanofuran dehydrogenase subunit a	571	F:catalytic activity	
2.1	2.1	2.9	Mhun_1181	v-type atp synthase subunit c	351	P:biological_process; C:cellular_component	

0	1.9	12.9	Mhun_1839	methyl-viologen-reducing hydrogenase delta subunit	62	F:receptor activity	EC:5.4.99.2
2.1	2.1	5.7	Mhun_1842	methyl-viologen-reducing hydrogenase delta subunit	140	P:methanogenesis; F:metal ion binding; P:electron transport chain; F:oxidoreductase activity; F:2 iron, 2 sulfur cluster binding; P:transport	EC:5.4.99.2
2.09	2.09	6.4	Mhun_0672	branched-chain amino acid aminotransferase	297	P:branched chain family amino acid biosynthetic process; F:branched-chain-amino-acid transaminase activity; F:D-alanine:2-oxoglutarate aminotransferase activity; F:lyase activity	
2.08	3.79	4.4	Mhun_0023	serine hydroxymethyltransferase	436	P:auxin biosynthetic process; P:protein folding; F:ATPase activity; P:response to stress; P:oxidation reduction; F:ATP binding; F:unfolded protein binding; F:2-alkenal reductase activity	EC:3.6.5.3
2.08	2.08	7.9	Mhun_3015	30S ribosomal protein s19e	140	C:ribosome; F:structural constituent of ribosome; F:RNA binding; P:ribosome biogenesis; P:translation	
2.08	2.08	7.4	Mhun_1601	50S ribosomal protein l7ae	122	F:structural constituent of ribosome; C:cytosolic small ribosomal subunit; P:translation	
2.02	15.95	41.7	Mhun_2147	methyl-coenzyme M methylreductase gamma subunit	252	F:iron-sulfur cluster binding; F:electron carrier activity; F:NADH dehydrogenase (ubiquinone) activity; F:iron ion binding; F:ferredoxin hydrogenase activity; P:ATP synthesis coupled electron transport; C:membrane	
2.01	2.6	2.7	Mhun_1981	formylmethanofuran dehydrogenase subunit c	332	C:cytoplasm; F:sulfurtransferase activity; F:protein binding; P:tRNA processing	
2	2	6.8	Mhun_2237	50S ribosomal protein l6p	176	P:glutamine metabolic process; P:cobalamin biosynthetic process; F:cobalamin-transporting ATPase	EC:2.7.2.1

						activity; F:amidase activity
2	2	5.3	Mhun_2229	adenylate kinase	190	F:transporter activity; P:transport

Supplemental Figures

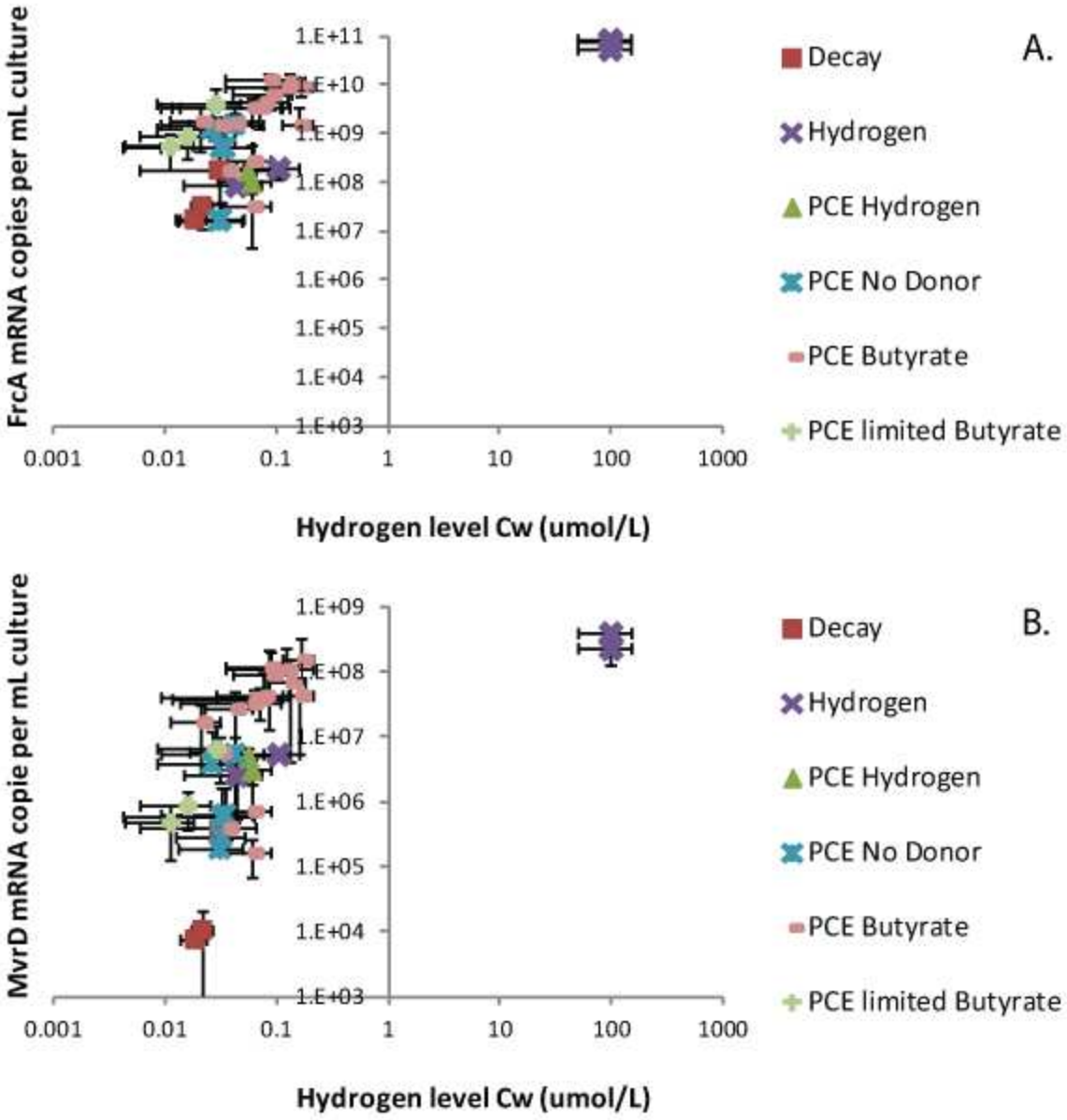


Figure S1. Pseudo-steady-state expression levels for subset of experiments listed in Table S2, compared with average dissolved hydrogen level (C_W) for FrcA (A) and MvrD (B). Experiments are grouped based on the type of electron donor and presence of PCE. X-error bars represent the standard deviation of average hydrogen levels over the course of the experiment. Y-error bars represent the standard deviation of PSS mRNA expression level over the course of the experiment.

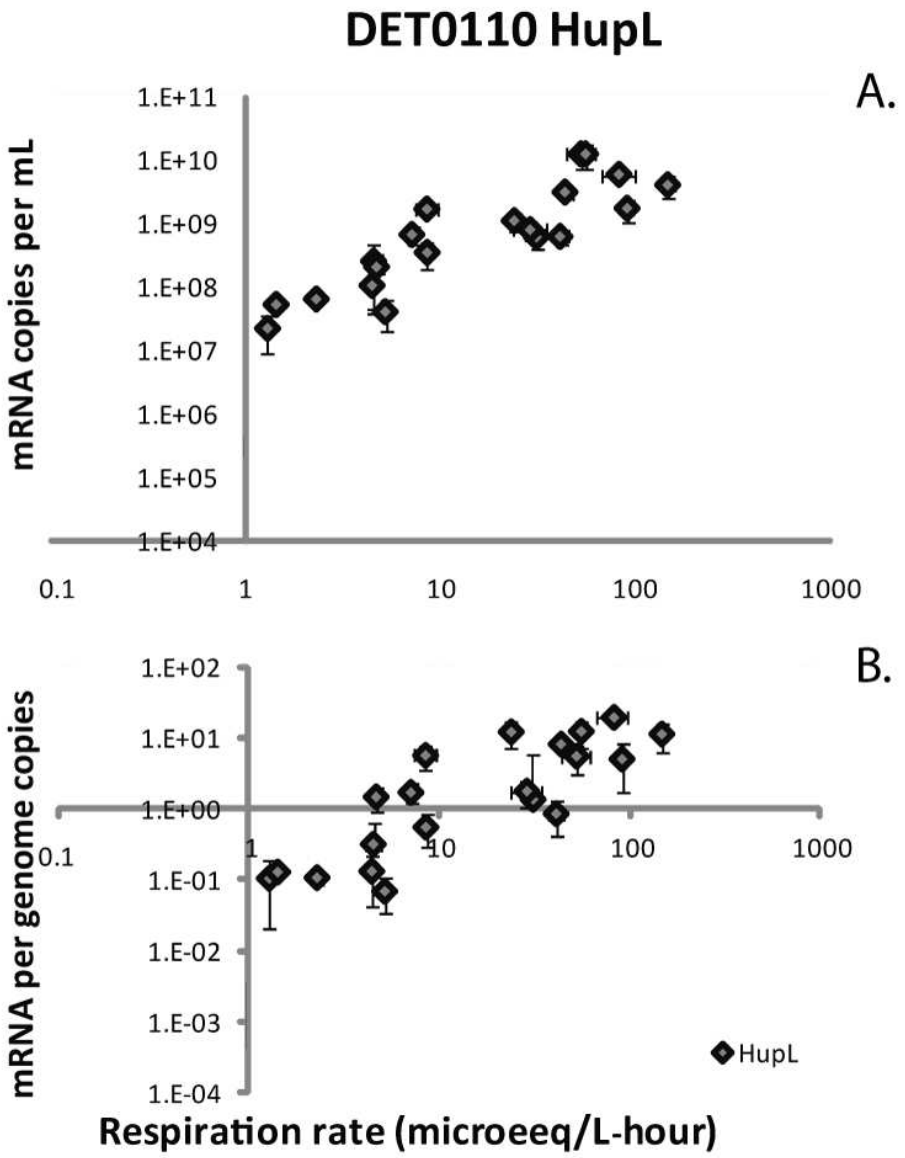
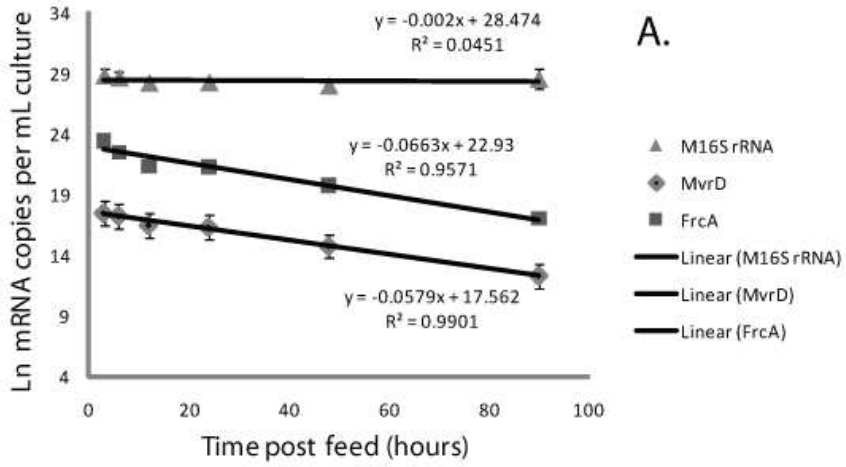
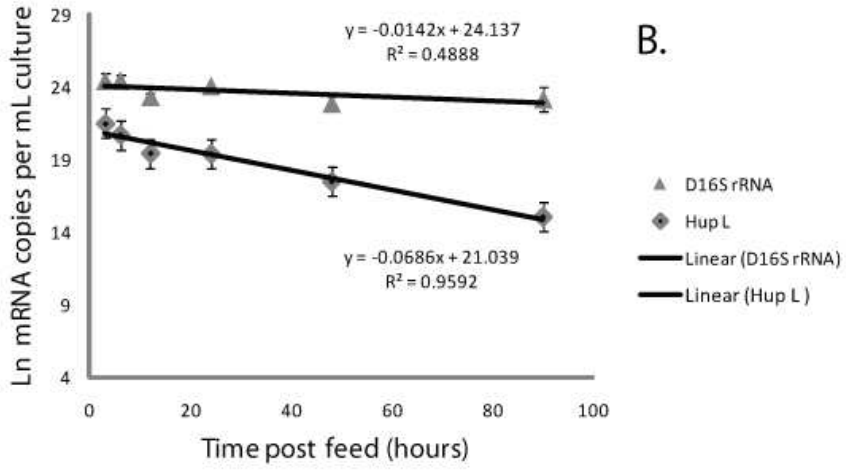


Figure S2. Pseudo-steady-state respiration rates vs. mRNA concentrations of specific *D. mccartyi* hydrogenase DET0110 HupL. Transcripts reported on a per mL (A), and a per 16S rRNA gene copy (B). Error bars represent standard error of average respiration rates between replicates (x-error bars) and standard deviations of PSS mRNA measurements over time for replicate reactors (y-error bars). For experimental conditions see Table S2.


A.
y = -0.002x + 28.474
R² = 0.0451
y = -0.0663x + 22.93
R² = 0.9571
y = -0.0579x + 17.562
R² = 0.9901
Ln mRNA copies per mL culture
Time post feed (hours)
M16S rRNA
MvrD
FrcA
Linear (M16S rRNA)
Linear (MvrD)
Linear (FrcA)


B.
y = -0.0142x + 24.137
R² = 0.4888
y = -0.0686x + 21.039
R² = 0.9592
Ln mRNA copies per mL culture
Time post feed (hours)
D16S rRNA
Hup L
Linear (D16S rRNA)
Linear (Hup L)

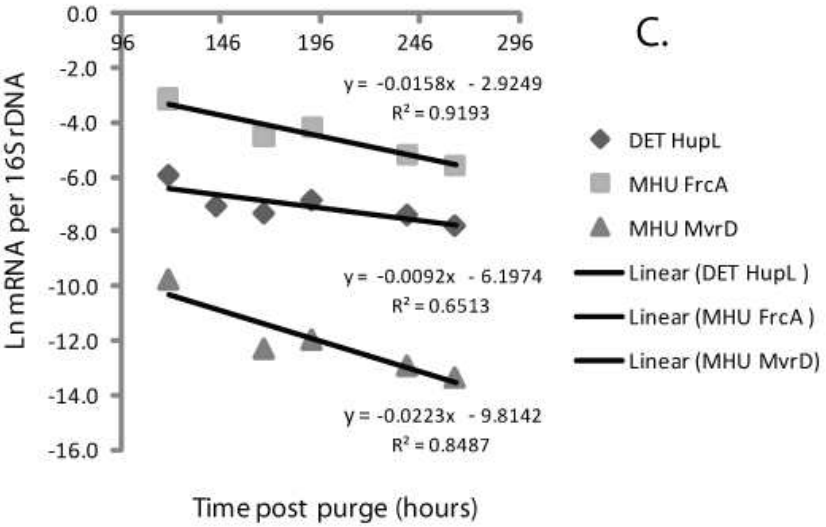

C.
y = -0.0158x - 2.9249
R² = 0.9193
y = -0.0092x - 6.1974
R² = 0.6513
y = -0.0223x - 9.8142
R² = 0.8487
Ln mRNA per 16S rDNA
Time post purge (hours)
DET HupL
MHU FrcA
MHU MvrD
Linear (DET HupL)
Linear (MHU FrcA)
Linear (MHU MvrD)

Figure S3. Active decay in transcript abundance post batch feed in PCE and butyrate cultures. Ln of transcript abundance for *M. hungatei* biomarker targets (A) and *D. mccartyi* biomarker targets (B) starting 3-6 hrs post feed plotted against time. Slopes indicate first-order decay coefficients. Error bars indicated the standard deviation of four samples per time point (n=4). Endogenous decay rates calculated post purge of end products (starting at 96 hrs post batch feed) (C). Ln of transcript abundance per 16S rRNA gene copy for each organism is plotted against time for calculating decay coefficients. Error bars indicate standard deviation of biological replicates (n=3).

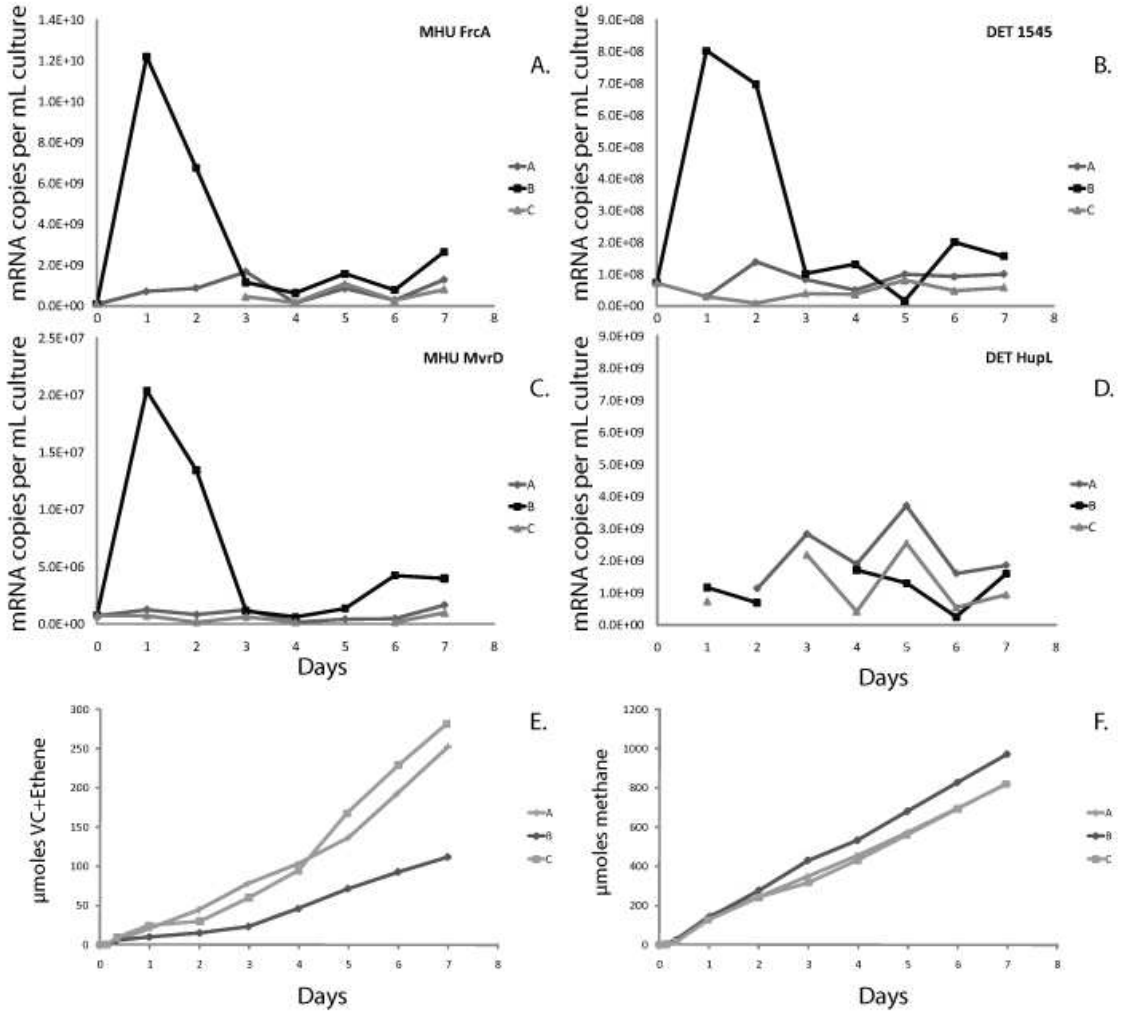


Figure S4. Expression time course of *D. mccartyi* and *M. hungatei* mRNAs during donor limited butyrate and PCE fed experiment (ratio of 0.5 to 1 ED to EA) (A-D). Each individual time course represents a biological replicate. Metabolites: PCE respiration products VC and Ethene (E), and methane (F) for these time courses. A syringed clog in replicate B caused decreased PCE addition, (butyrate syringe was not affected). Methane produced during these experiments is the result of acetoclastic and hydrogenotrophic methanogenesis.

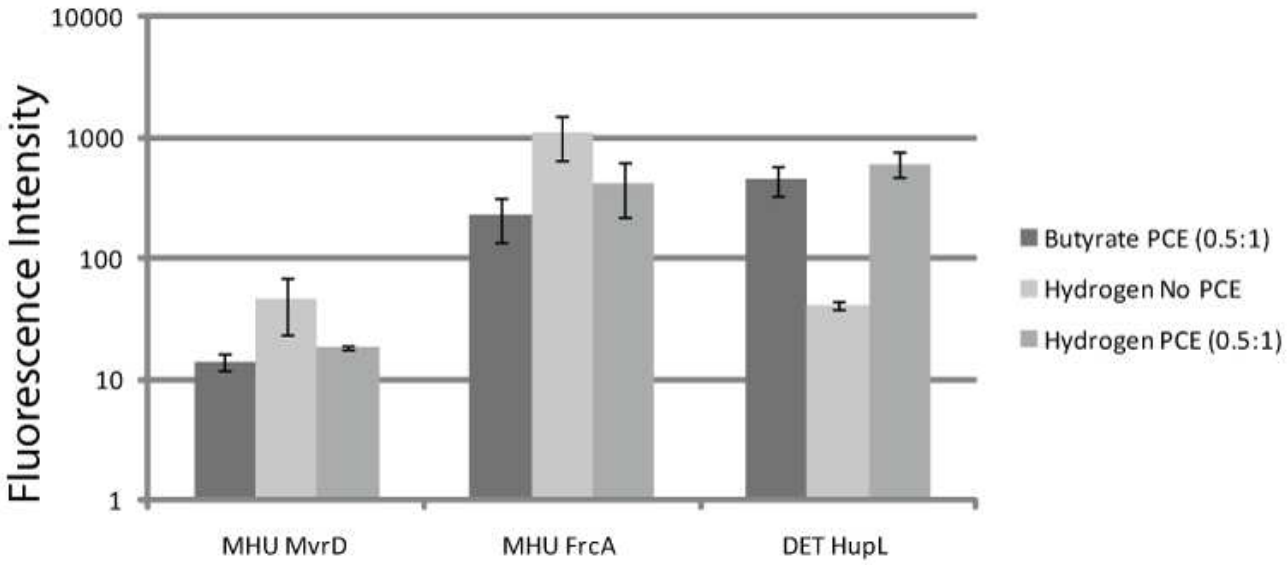


Figure S5. Absolute intensity based on mixed culture microarray experiments. Error bars indicate the average intensity measured from 6 to20 replicate probe spots. Data are from experiments with and without PCE added.

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